African Journal of Biotechnology Vol. 6 (12), pp. 1450-1454, 18 June 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Biodegradation of penicillin-G wastewater using *Phanerochate chrysosporium* – An equilibrium and kinetic modeling

P. Mullai[1]* and S. Vishali[2]

[1]Department of Technology, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India.
[2]Department of Chemical Engineering, SRM Engineering College, SRM University, Katankulathur-603 203, Chengalpet District, Tamil Nadu, India.

Accepted 25 April, 2007

An attempt was made in the present study to find out the biodegradation of the penicillin-G wastewater for the various operational conditions such as, initial substrate concentrations (13000, 10000, 6000, 4000 and 2000 mg of COD/l), agitation, addition of nutrients (glucose and ammonium chloride) and biomass dosages (2, 3, 4 and 5 g) in batch reactor using immobilized cells of *Phanerochate chrysosporium,* a white rot fungi. The highest COD removal efficiency was found at the initial substrate concentration of 2000 mg COD /l, under static condition using 4 g of biomass in the absence of nutrients i.e., carbon and nitrogen sources. The Langmuir and Freundlich adsorption models fitted well with the equilibrium data of the process studied. It was also observed that the experimental kinetic data followed the first order rate expression.

Key words: Penicillin-G wastewater*, Phanerochate chrysosporium*, equilibrium, kinetic study.

INTRODUCTION

When compared to other industries greater emphasis is placed on pharmaceutical industries due to increasing needs of drugs for the escalating human population. The demand for pharmaceutical products in the area of human and animal health care during 2001 was estimated at Rs. 3500 crore in India (Rawlins, 1995). There are over 15000 drug manufacturing units in the country and the majority of them are small scale units. The pharmaceutical and fine chemical industries are the second largest source of effluents in India and generate about 43 tons of chemical oxygen demand (COD) per day**.** The wastewater generated from the pharmaceutical industries contains significant levels of aliphatic organic solvents which contribute alarmingly for the BOD/COD content of the wastewater (Mullai, 2002)**.**

Even though many physico-chemical methods are available for the treatment of penicillin-G wastewater, they could not be implemented as a result of high cost, emission of toxic substances and formation of sludge. In recent years, a white rot fungus, *Phanerochate chrysosporium* has attracted considerable attention due to its ability to degrade a variety of organic pollutants by the extracellular peroxidase enzymes. The use of *P. chrysosporium* as a potential microorganism for the biodegradation of polychlorinated biphenyls (Eaton, 1985), a large number of explosives most notably TNT (Fernando et al., 1990), paper mill bleach plant effluent (Fukui, 1992) and spentwash (Fahy et al., 1997) was reported. But no research work seemed to have been carried out on the use of *P. chrysosporium* in the degradation of penicillin-G wastewater. This paper deals with the equilibrium and kinetic study of biodegradation of penicillin-G wastewater using the immobilized cells of *P. chrysosporium*.

*Corresponding author. E-mail: sammullai@yahoo.com.

Table 1. The physicochemical characteristics of penicillin-G wastewater.

Parameters	Concentration
PH	5.5 - 6.5
Colour	Yellowish
Odour	Fruity smell
BOD (mg/l)	5000 - 9000
COD (mg/l)	15000 - 25000
Ammonia-nitrogen (mg/l)	300 - 500
Total phosphate (mg/l)	70 - 200
Acidity as acetic acid (mg/l)	300 - 500
Alkalinity as $CaCO_3$ (mg/l)	1000 - 2000
Sulphate (mg/l)	3000 - 4000
Sulphide (mg/l)	50 - 150
Total solids (mg/l)	1000 - 3000
Temperature (°C)	30 - 45

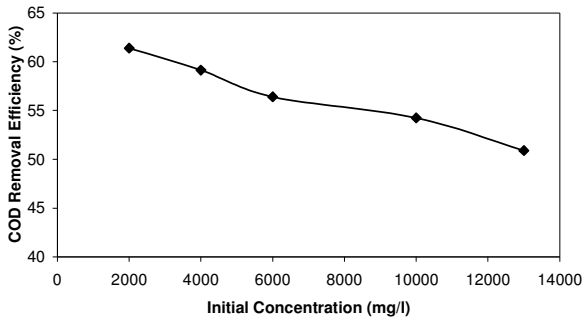

Figure 1. Effect of initial concentration on COD removal efficiency using 4 g of immobilised *P. chrysosporium* at natural pH.

MATERIALS AND METHODS

Sample collection

The effluent was collected from the penicillin-G manufacturing unit, Cuddalore, Tamilnadu, India. The physico-chemical characteristics of the samples are given in Table 1.

Microorganism and growth conditions

Pure culture of *P. chrysosporium*, a fungal strain was obtained from National Chemical Laboratory (NCL), Pune, India. Stock cultures of the strain were transferred to sterile slants of potato dextrose agar (PDA) media. Fungal cells were cultivated at 30°C for 7 days. The biomass slants were transferred to 50 ml of appropriate sterile growth liquid media containing potato and dextrose. The cell suspension was then separated, centrifuged, dried, homogenised and stored at 4°C for further use.

Potato dextrose agar medium was prepared by taking 200 g of peeled and sliced potato with one litre of distilled water and steamed for 30 min. The extract was made into final volume of one litre. Thereafter, 20 g of dextrose, 0.1 g of yeast extract and 20 g of agar were added to it.

Immobilisation of *P. chrysosporium*

The cells were immobilized using 8% sodium alginate in 2% calcium chloride solution. The beads were cured at 4°C over a night. The immobilised beads were rinsed twice in distilled water before use.

Batch study

Batch experiments were carried out in 500 ml Erlenmeyer Flasks using the immobilized beads of *P. chrysosporium* containing 300 ml of the effluent. The flasks (bioreactors) were gently agitated at room temperature on a shaker at 150 rpm for ten days. The samples were taken from the bioreactor at every 24 h and analysed for the chemical oxygen demand (COD) following the procedure specified in APHA Standard Methods (1995). The experiments were carried out to study the effect of operational parameters such as different initial concentrations, shaking, addition of nutrients and the biomass dosages. For the determination of adsorption isotherms, 4 g of biosorbent was used at five different concentrations such as 13000, 10000, 6000, 4000 and 2000 mg COD/l.

RESULTS AND DISCUSSION

Effect of initial concentrations

For the five different initial concentrations of penicillin-G wastewater such as 13000, 10000, 6000, 4000 and 2000 mg COD/l, the steady state values of COD removal efficiencies were 50.9, 54.25, 56.4, 59.15 and 61.4%, respectively, using 4 grams of *P. chrysosoporium* under static condition at the end of 10^{th} day (Figure 1). The COD removal efficiency was found to decrease with increase in the initial concentration of the wastewater. This trend might be due to the lack of available active sites on the immobilized surface and also substrate inhibition as reported by Mullai and Sathian (2003) in their work on treatment of spentwash using immobilized *Paecilomyces variotii.*

Effect of biomass

For the initial concentration of 2000 mg COD/l, the COD removal efficiencies, at four different dosage values such as 2, 3, 4 and 5 g, were 56.2, 57.9, 61.4 and 58.6%, respectively at the end of 10th day (Figure 2). With the increase in biomass loading from 2 to 4 g, the COD removal efficiencies increased due to the availability of more active adsorbing sites. Further increase of biomass dosage to 5 g brought down the COD removal efficiency. It was due to greater crowding in the suspension at higher adsorbent dosage, the active sites of the adsorbents might not be energetically homogeneous for approaching peni-

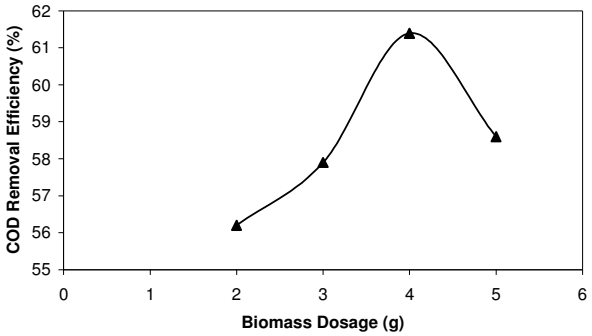

Figure 2. Effect of biomass loading on COD removal efficiency.

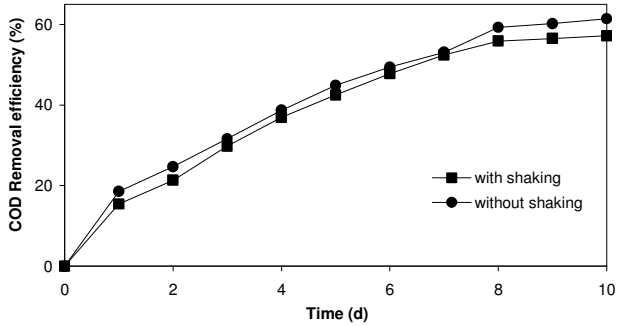

Figure 3. Effect of shaking on COD removal efficiency.

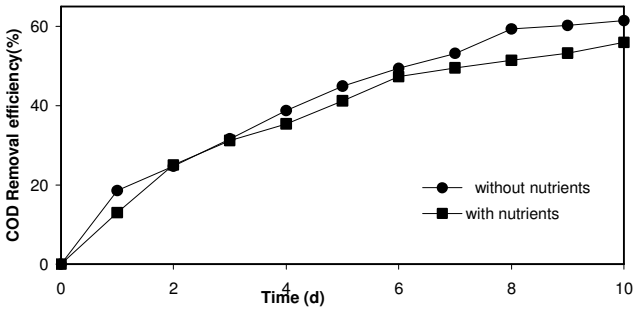

Figure 4. Effect of nutrients on COD removal efficiency.

cillin-G wastewater.

Effect of shaking

In the experiments carried out under 'shaking' and 'static' conditions with the initial concentration of 2000 mg COD/l using *P. chrysosporium* the equilibrium COD removal efficiencies obtained at the end of 10th day for the said two conditions were 57.2% and 61.4%, respectively (Figure 3). The possibility of higher levels of dissolved oxygen in agitated culture than the stationary culture probably caused the inactivation of lignin peroxidase (LiP) as reported by Manimegalai (1999).

Effect of nutrients

The initial substrate concentration of 2000 mg COD/l effluent was treated "with" and "without" addition of 5 g /l of glucose as carbon source and ammonium chloride as nitrogen source each. At the end of the 10th day, the equilibrium COD removal efficiencies obtained were 55.9% and 61.4% for the case of addition of nutrients and without the addition of nutrients, respectively (Figure 4). The nutrient does not have any influence on the treatment, which might be due to the inability of the fungus to utilize the nutrients. Fu and Vijayaraghavan (2000) stated similar results and concluded that the degradation of dyes using *P. chrysosporium* was good under the nutrient limitations may be due to the secretion of LiP an extracellular enzyme.

Equilibrium study

The equilibrium of the process is often described by fitting the experimental points with models usually for the representation of isotherm adsorption equilibrium. The amount of material adsorbed is determined as a function of concentration at constant temperature, and the resulting function is known as adsorption isotherm which provides an appropriate estimate of the adsorption capacity and intensity by adsorbents.

Langmuir isotherm

The basic assumption of the Langmuir theory is that adsorption takes place at specific sites within the adsorbent and beyond the saturation value; no further adsorption can takes place. The Langmuir isotherm model is expressed as:

$$q_e = \frac{Q^o b C_e}{1 + bC_e} \qquad \ldots (1)$$

where q_e (mg/g) and C_e (mg/l) are the amount of adsorbed pollutant per unit weight of biosorbent and unadsorbed pollutant concentration in effluent at equilibrium, respectively. Q^o (mg/g) is the maximum amount of the pollutant adsorbed per unit weight of biosorbent to form a complete monolayer on the surface and b (l/mg) is a constant related to the affinity of the binding sites.

From the slope and intercept of the linearized plot of $1/q_e$ versus $1/C_e$, the isotherm constants, Q^o and b were

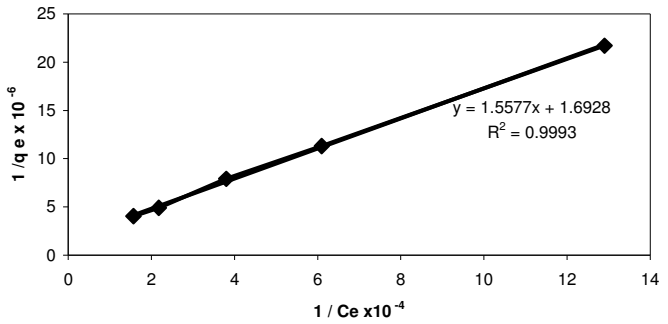

Figure 5. Langmuir isotherm for different concentrations.

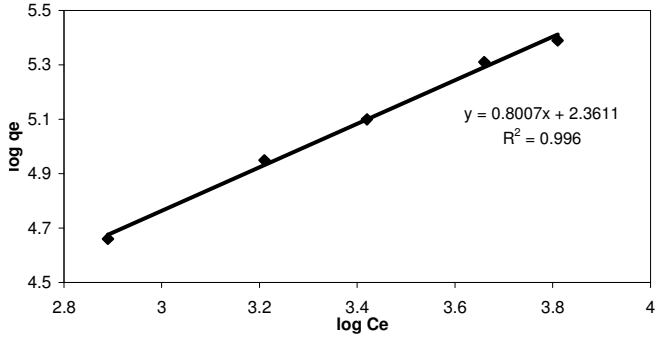

Figure 6. Freundlich isotherm for different concentrations.

calculated (Figure 5) and the values were 0.591 and 1.087 respectively. The essential characteristics of Langmuir isotherm can be described by a separation factor (Hall et al., 1996), which is defined by, $R_L = \frac{1}{1+bC_i}$. Since the value of R_L lies between 0 and 1, the reported isotherm represents the favourable adsorption. Moreover, the higher correlation coefficient value (R^2 = 0.9993) confirmed the suitability of the model.

Freundlich isotherm

The Freundlich isotherm is based on the heterogeneous surface is expressed as:

$$q_e = K_F C_e^{1/n} \quad \ldots (2)$$

where K_F and n are the Freundlich constants and the indicators of adsorption capacity and adsorption intensity, respectively. Freundlich constants were determined from the linear plot of log q_e versus log C_e (Figure 6) and their values were 1.25 and 230, respectively. According to Treybal (1988), the values n >1 represent favourable Freundlich isotherm adsorption condition and the same was obtained in the present investigation.

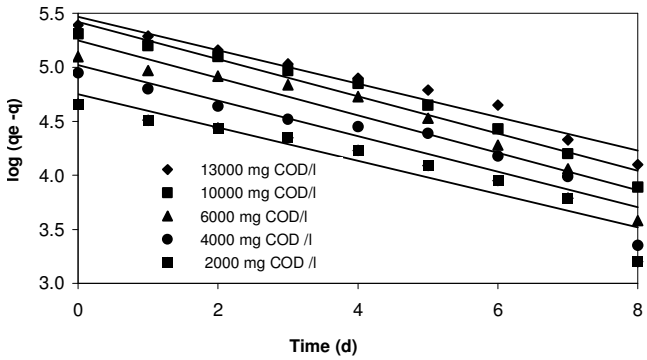

Figure 7. First order for different concentrations.

Table 2. Theoretical and experimental q_e values.

Initial concentration (mg COD/l)	q_e (exp)	q_e (theo)
2000	5.47	5.39
4000	5.42	5.31
6000	5.25	5.1
10000	5.02	4.95
13000	4.75	4.66

Kinetic study

The first order rate constant for adsorption of different initial substrate concentrations was studied using Lagergren equation:

$$\frac{dq}{dt} = K_{1,ad}(q_e - q) \quad \ldots (3)$$

q, the amount adsorbed by sorbent (mg/g) at time was calculated as,

$$q = \frac{(C_o - C_f)V}{W} \quad \ldots (4)$$

The integrated form of Equation (3) is

$$\log(q_e - q) = \frac{\log q_e}{2.303} - K_{1,ad}t \quad \ldots (5)$$

Linear plots of log (q_e-q) Vs t indicated the applicability of the above equation and the first order of the process (Figure 7). The theoretical q_e values were found to be in good agreement with the experimental q_e values (Table 2). Annadurai et al. (2000) reported similar results on the adsorption and biodegradation of phenol by chitosan immobilized *Pseudomonas putida*.

Conclusions

The immobilized *P. chrysosporium* performed well in the degradation of penicillin-G wastewater. The COD removal efficiency increased with increase in treatment time. The COD removal efficiency was highest in the static condition, without the addition of nutrients for the initial concentration of 2000 mg COD /l using 4 g of *P. chrysosporium*. The equilibrium data fitted very well with both the Langmuir and Freundlich adsorption isotherms. The linearity observed in the Lagergren plot suggested the first order nature of adsorption.

REFERENCES

Annadurai G, Rajesh Babu S, Mahesh KPO (2000). Adsorption and biodegradation of phenol by chitosan immobilized *P.putida.* Bio. Pro. Eng. 22: 493-499.

APHA (1995). Standard methods for the examination of waste and wastewater, 16th edition, American Public Health Associations, New York.

Eaton DC (1985). Mineralisation of polychlorinated biphenyls by *Phanerochaete chrysosporium.* Enzy. Microbiol. Technol. 7: 194-196.

Fahy V, Fitzgibbon FJ, Mcmullan G, Singh D, Marchant R (1997). Decolourisation of molasses spentwash by *Phanerochaete chrysosporium,* Biotechnol. Lett. 19: 97-99.

Fernando T, Bumpus JA, Aust SD (1990). Biodegradation of 2, 4, 6 trinitrotoluene by *Phanerochaete chrysosporium.* Appl. Environ. Microbiol. 56: 1666-1671.

Fu Y, Viraraghavan T (2000). Fungal decolourization of dye wastewater. Biores. Tech. 79: 251-262.

Fukui H (1992). Dechlorination and detoxification of bleach effluent by *Phanerochaete chrysosporium.* J. Biotechnol. 24: 267-275.

Hall KR, Eagleton L, Vermeulen T (1996). Pore and solid diffusion kinetics in fixed bed adsorption under constant pattern conditions. Ind. Engg. Chem. Fund. 5: 221-223.

Manimekalai R (1999). Studies on biodegradation of toxic compounds using P.chrysosporium. PhD dissertation, Indian Institute of Technology, Chennai, India.

Mullai P (2002). Industrial wastewater treatment using a HUASB reactor. PhD dissertation, Annamalai University, Tamil Nadu, India.

Mullai P, Sathian S (2003). Treatment of Spentwash using Immobilised *Paecilomyces variotii*. Proceedings of an international congress held at Annamalai University, Annamalai Nagar, p. 77.

Rawlins EA (1995). Bentely's textbook of pharmaceuticals, First Edition, All India publishers and distributors, India.

Treybal RE (1988). Mass transfer operations, Tenth Edition, Mc Graw Hill, New York.